tsRTarget

Requirements:

- Linux system, enough disk space and Ram depending on the size of RNA deep sequencing data. (Tested system: ubuntu 12.04 LTS, ubuntu 16.04 LTS)

Installation

- Download tsRTarget pipeline package from https://github.com/zhlingl/tsRFun

```
wget https://github.com/zhlingl/tsRFun/archive/refs/heads/main.zip
```

- Download necessary software, packages and reference databases as listed below:
    - The CLIP Tool Kit (CTK) (https://zhanglab.c2b2.columbia.edu/index.php/CTK_DocumentationTested version:1.1.3)
    - RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/)
    - BLAST(https://blast.ncbi.nlm.nih.gov/Blast.cgi)(Tested version:2.10.1)
    - Reference database (See lists and download link of all pre-compiled species' databases in Pre-compiled Databases Instruction)
- Download tsRTarget pipeline package
    - Install tsRTarget from https://github.com/zhlingl/tsRFun
        - Unpack tsRTarget package

```
 unzip main.zip
```

        - Attach the tsRTarget directory to your PATH:

```
echo 'export PATH=$PATH:your_path_to_tsRFun-master/tsRTarget' >> ~/.bashrc.
chmod 755 your_path_to_tsRTarget-master/tsRTarget/tsRTarget.sh
```

    - Install CTK
        - Unpack czplib-1.0.x.zip

```
unzip czplib-1.0.x.zip
mv czplib-1.0.x /usr/local/lib/czplib
```

        - Add the library path to the environment variable, so perl can find it.

```
export PERL5LIB=your_path_to_czplib
```

        - Download CTK code and likewise decompress and move to whatever directory you like (as an example, we use /usr/local/)

```
unzip ctk-1.0.x.zip
mv ctk-1.0.x /usr/local/CTK
```

        - Attach the CTK directory to your PATH:

```
echo 'export PATH=$PATH:your_path_to_CTK'
```

    - Install RNAhybrid
        - Unpack RNAhybrid-2.1.2.tar.gz.

```
wget https://bibiserv.cebitec.uni-
bielefeld.de/applications/rnahybrid/resources/downloads/RNAhybrid-2.1.2.tar.gz
tar -xzvf RNAhybrid-2.1.2.tar.gz
cd /path/to/ RNAhybrid-2.1.2
./configure
make
```

```
make install
cd bin/
```

- Attach the bedtools directory to your PATH:

```
echo 'export PATH=$PATH:your_path_to_RNAhybrid' >> ~/.bashrc
```

- Start a new shell session to apply changes to environment variables:

```
source ~/.bashrc
```

- Install BLAST
  - Unpack ncbi-blast-2.10.1+-x64-linux.tar.gz from https://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/.

```
tar -zxvf ncbi-blast-2.10.1+-x64-linux.tar.gz
```

  - Attach the samtools directory to your PATH:

```
echo 'export PATH=$PATH:your_path_to_BLAST' >> ~/.bashrc
```

  - Start a new shell session to apply changes to environment variables:

```
source ~/.bashrc
```

- Download tsRFun pipeline package
  - Test if everything is installed properly:

```
perl -v
bash tsRTargt.sh -h
ctk -h
RNAhybrid -h
blastn -h
```

- If you get any error messages you should install the software or perl modules once again.

Script description

- The input files of tsRTarget.sh are:

```
  Options:
    -i <inputfile>      Input could be:
                 a .fastq/.fq or .fasta/.fa file.
    -t data type clear/clash or CLIP (iclip/eclip/par-clip/hits-clip)
    -o outputdir  address of annotation results
    -a index_adress
    -m mismatch  default=1
    -w min tsRNA_length word_size default=14
    -n max tsRNA_length word_size default=40
    -g min target_length  default=10
    -h max -g min target_length default(70 for clear clash data; 140 for clip data)
    -s matched-length  default=6
    -e gapopen  default=1
    -P Collapse PCR duplicates default=T
    -S seed T/N  default=T
    -R seed start default=2
    -E seed end default=8
    -M Min Free Energy  default=-10
    -c match_clip the word_size of RNA-RNA target; only used for CLIP seq data,
Others:
    -h          print this usage message
```

Example of use:

```
bash tsRTarget.sh -i example.fa -t clear -o example_result -a hg38_index
```